Microbial biosorbents: Meeting challenges of heavy metal pollution in aqueous solutions

Rani Gupta*,#, Prerna Ahuja†, Seema Khan*, R. K. Saxena* and Harapriya Mohapatra*

*Department of Microbiology, University of Delhi South Campus, Benito Juarez Road, New Delhi 110 021, India
†Ramlal Anand College, Benito Juarez Road, University of Delhi South Campus, New Delhi 110 021, India

Heavy metal pollution in the aquatic system has become a serious threat today. The chemical processes that exist are not economical for treating a large volume of water bodies of dilute metal concentration. In this endeavour, microbial biomass has emerged as an option for developing economic and ecofriendly wastewater treatment processes. Non-living and dead microbial biomass may passively sequester metal(s) by the process of biosorption from dilute solutions. This biosorption technology has advantages of low operating cost, is effective in dilute solutions and generates minimum effluent. Here the dead microbial biomass functions as an ion exchanger by virtue of various reactive groups available on the cell surface such as carboxyl, amine, imidazole, phosphate, sulfhydryl, sulfate and hydroxyl. The process can be made economical by procuring natural bulk biomass or spent biomass from various fermentation industries. The performance of a biosorbent can further be improved by various physical and chemical treatments. The pretreatments modify the cell surface either by removing or masking the groups or exposing more metal binding sites. Immobilized biomass of these microbes offers the continuous sorption–desorption system in a fixed bed reactor. Various commercial microbial biosorbents available are AlgaSorb, AMT-Bioclaim and Bio-fix. The economics of these sorbents merit their commercialization, over chemical ion exchangers.

HEAVY metal(s) are widespread pollutants of great environmental concern as they are non-degradable and thus persistent[1]. It is well perceived that there is a permissible limit of each metal, above which they are generally toxic[2,3] and some are even hazardous. Globally, in developed countries, pollution of the aquatic system is controlled by the union under the framework 'Dangerous Substances Directive' which has led to certain environmental protection acts and regulations enforced by environmental agencies. Consequently, all effluents need to be assessed and require integrated pollution documentation before their final discharge. Looking into the increased environmental awareness, even in developing countries like India, wastewater treatment is of utmost importance. The degree of treatment may range from a main process for seriously polluted industrial waste to a polishing process for removing the trace concentrations which remain after the main treatment. The conventional processes used for effluent treatment are precipitation as hydroxides/sulphides, oxidation/reduction and ion exchange. The processes are expensive and not ecofriendly[4–6]. Further, the major disadvantage with conventional treatment techniques is the production of sludge. As a result, an aquatic problem is changed into solid disposal problem. Therefore, amongst the chemical adsorbents only ion exchange resins were considered as the option for remediation with least ecological problem. However, chemical resins are expensive and the increasing demand of eco-friendly technologies has led to the search of low-cost alternatives which could be considered as single use materials. In this light, biological materials have emerged as an ecofriendly and economic option[7]. For a long time, peat has occupied the place of prominence among biosorbents, but since it is not available everywhere, microbial biomass is the other option. Microbial biomass can passively bind large amounts of metal(s), a phenomenon commonly referred to as biosorption[8–11], thus providing a cost-effective solution for industrial wastewater management[12]. However, on prolonged contact with the metal-bearing solution, the living biomass is also able to sequester metal intracellularly by an active process called bioaccumulation. Biosorption is possible by both living and non-living biomass; however, bioaccumulation is mediated only by living biomass[13–15]. Further, bioaccumulation is a growth dependent process and it is difficult to define a variety of effluents in contrast to biosorption which is growth-independent. Thus, microbial biomass can be used and exploited more effectively as biosorbents rather than accumulation.

As early as in 1986, at a meeting organized by the Solvent Engineering Extraction and Ion Exchange Group of the Society of Chemical Industry at UK, biosorption was regarded as an emergent technology[16]. Since then a number of centres all over the world have

#For correspondence. (e-mail: micro@dusc.ernet.in)

been engaged in the area of biosorption with precise goals of identifying potential biomass(es)[17–21].

Till date, research in the area of biosorption suggests it to be an ideal alternative for decontamination of metal-containing effluents. Biosorbents are attractive since naturally occurring biomass(es) or spent biomass(es) can be effectively utilized. Besides this, biosorption offers advantages of low operating cost, minimizes the volume of chemical and/or biological sludge to be disposed, is highly efficient in dilute effluents and has no nutrient requirements. These advantages have served as potential incentives for promoting biosorption as a viable clean-up technology for heavy metal(s) pollution[22].

Biosorption is a rapid phenomenon of passive metal sequestration by the non-growing biomass[23–25]. Results are convincing and binding capacities of certain biomass(es) are comparable with the commercial synthetic cation exchange resins[26]. Biosorption mainly involves cell surface complexation, ion exchange and microprecipitation[5,27,28]. Different microbes have been found to vary in their affinity for different heavy metal(s) and hence differ in their metal-binding capacities. Some biomass(es) exhibit preference for certain heavy metal(s) whereas others do not show any specific binding and are broad range[29–31].

The role of various groups of micro-organisms in the removal and recovery of heavy metal(s) by biosorption has been well reviewed[1,4,6,26,31,32]. A large number of micro-organisms belonging to various groups, viz. bacteria, fungi, yeasts, cyanobacteria and algae have been reported to bind a variety of heavy metals to different extents. Volesky and Holan[12] have presented an exhaustive list of microbes and their metal-binding capacities.

Among micro-organisms, fungal biomass offers the advantage of having a high percentage of cell wall material which shows excellent metal-binding properties[27,33,34]. Many fungi and yeast have shown an excellent potential of metal biosorption, particularly the genera *Rhizopus, Aspergillus, Streptoverticillum* and *Saccharomyces*[35–41]. Among bacteria, *Bacillus* sp. has been identified as having a high potential for metal sequestration and has been used in commercial biosorbent preparation[17]. Besides there are reports on the biosorption of metal(s) using *Pseudomonas* sp, *Zoogloea ramigera* and *Streptomyces* sp.[42–46]. Among photoautotrophs marine algae became the candidate of interest due to bulk availability of their biomass from water bodies[5]. *Sargassum natans* and *Ascophyllum nodosum* in this group have shown very high biosorptive capacities for various metal(s)[19,47–49]. Besides marine algae, there are reports on binding of heavy metal(s) to green algae, viz. *Chlorella* sp.[6,50–54] and cyanobacteria[31,50,55].

The sorption capacity is evaluated by the sorption isotherms described by Langmuir and Freundlich[56,57]. The uptake of metal by two biosorbents must be compared at the same equilibrium concentration. The adsorption is easy to understand when it refers to the single metal situation; however in a multi-ion situation which is generally encountered in effluents, the assessment of sorption analysis becomes complicated. Most of the work exists with single metal solution and a realistic approach would be to infer results in mixed metal solutions, at extreme pH and variable metal concentration.

Metal binding sites

Performance of the biosorbent depends on the ionic state of the biomass and thus like the synthetic resins, biosorbents can be prepared with different ionic forms such as protonated (H^+ forms) or saturated with cations such as Na^+, Ca^{2+}, Mg^{2+}, etc. by pretreating the biomass with mineral acids, bases and/or salts. The treatment varies with the biomass type and the metal species to be biosorbed. To develop an efficient biosorbent and its reuse by subsequent desorption processes, knowledge of the mechanism of metal binding is thus very important. Metal binding appears to be at least a two-step process where the first step involves a stoichiometric interaction between the metal and the reactive chemical groups in the cell wall and the second step is an inorganic deposition of increased amounts of metal(s)[58]. All the metal ions before gaining access to the plasma membrane and cell cytoplasm come across the cell wall. The cell wall consists of a variety of polysaccharides and proteins and hence offers a number of active sites capable of binding metal ions[49]. Thus it is regarded as a complex ion exchanger similar to a commercial resin. Difference in cell wall composition among different groups of micro-organisms, viz. algae, bacteria, cyanobacteria and fungi and the intra group differences can thus cause significant differences in the type and amount of metal ion binding to them. Among the photoautotrophs, eukaryotic algal cell walls are mainly cellulosic. According to Crist *et al.*[59], the potential metal binding groups in this class of microbes are carboxylate, amine, imidazole, phosphate, sulfhydryl, sulfate and hydroxyl. Of these, amine and imidazoles are positively charged when protonated and may build negatively charged metal complexes[31]. The cell walls of brown algae contain fucoidin and alginic acid. The alginic acid offers anionic carboxylate and sulfate sites at neutral pH. The fresh water forms contain galacturonic acid and its polymer pectin which also has anionic sites to which metal(s) can bind by electrostatic attractions[59,60]. The amino and carboxyl groups, and nitrogen and oxygen of the peptide bonds are also available for coordination bonding with metal ions such as lead (II), copper (II) or chromium (IV). Such bond formation could be accompanied by displacement of protons and is dependent in part on the extent of protonation which is determined by the pH (refs 6, 28, 51, 61). The various groups involved in

metal binding have been discerned using the modification/blocking of the groups[62]. Carboxyl groups were suggested to be involved in binding Cu^{2+} and Al^{3+} in algal species as blocking of carboxyl groups by esterification led to a decrease in metal binding[63].

Cell walls of bacteria and cyanobacteria are principally composed of peptidoglycans which consist of linear chains of the disaccharide *N*-acetylglucosamine-β 1,4-*N*-acetylmuramic acid with peptide chains. Cell walls of gram-negative bacteria are somewhat thinner than the gram-positive ones and are also not heavily cross-linked. They have an outer membrane which is composed of an outer layer of lipopolysaccharide (LPS), phospholipids and proteins[64]. Gourdon *et al.*[25] compared the Cd^{2+} biosorption capacities of gram-positive and gram-negative bacteria. Glycoproteins present on the outer side of gram-positive bacterial cell walls have been suggested to have more potential binding sites for Cd^{2+} than the phospholipids and LPS and hence are responsible for the observed difference in capacity. Carboxyl group modification caused a marked reduction in metal uptake by *B. subtilis*[58]. However, amine modification did not alter the metal uptake by the bacterium. In *B. subtilis*, teichoic acid[58] and in *B. licheniformis*, teichoic acid and teichouronic acid[65] were found to be the prime sites for metal binding. In *E. coli* K12, peptidoglycan was found to be a potent binder of most of the metal(s) tested and carboxylate groups were the principal components involved in metal binding[65]. The phosphoryl groups of the LPS and phospholipids have been demonstrated to be the most probable binding sites for metal cations in the *E. coli* outer membrane[66,67]. In purified cell envelopes of *E. coli* K12, most of the metal deposition occurred at the polar head regions of constituents membranes or along the peptidoglycan[68]. In *Streptomyces longwoodensis*, phosphate residues were suggested to be the primary constituents responsible for uranium binding[59].

In fungi, the mechanism of uranium uptake by *Rhizopus arrhizus* has been well worked out[11]. The uranium biosorption involves amine nitrogen of chitin crystallites and takes place in a sequence of events. Other studies in fungi have implicated PO_4^{3-} and COO^- groups of the cell wall as the primary binding sites[62].

Extracellular polymeric substances have also been shown to bind metal ions selectively with high metal accumulating potentials[69]. These polymers have anionic potentials and hence bind metal cations and sometimes form capsules or loose aggregates around cells[8,45,46].

A very unique mechanism of metal uptake was reported in *Citrobacter* sp.[70–75]. *Citrobacter* sp. showed a very high U^{6+}, Cd^{2+}, Cu^{2+} and Pb^{2+} removal from solution supplemented with glycerol 2-phosphate. The mechanism of uptake involved a phosphatase-mediated cleavage of glycerol 2-phosphate to release HPO_4^{2-} which precipitated metal on the surface as insoluble metal phosphate, e.g. $CdHPO_4$. This process appears to have a potential application where phosphate-containing organic substrates are present in metals and/or radionuclides containing effluents.

The metal binding in many studies has been suggested to be an ion exchange phenomenon. Greene, McPherson and Darnall[69] demonstrated the binding of Cu^{2+}, Pb^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+} and Cr^{3+} to *Spirulina platensis* to be accompanied by the liberation of protons suggesting an ion exchange reaction. Similar results were obtained in Cd^{2+}, $Cu^{2+}$and Zn^{2+} binding by previously protonated biomass of *Sargassum fluitans* where the metal binding was coupled with the release of H^+ ions[70]. We have also obtained similar results where copper and zinc binding was accompanied by release of large amounts of magnesium ions in *Oscillatoria anguistissima*[71,72]. The dominant mechanism of Cu^{2+} biosorption by *Ecklonia radiata* as ion exchange mechanism involving exchange of Ca^{2+} and Mg^{2+} ions present in their cell walls has been reported by Matheickal *et al.*[76]. Williams and Edyvean[77] have observed a concomitant release of Ca^{2+} ions during the biosorption of Ni^{2+} ions by the brown seaweed *Ecklonia maxima*. Thus biosorbents can be viewed as natural ion exchange materials that contain weak acidic and basic groups.

The mechanism of metal binding is not well understood due to the complex nature of microbial biomass, which is not readily amenable to instrumental analysis[49]. However localization of metal(s) has been carried out using electron microscopic and X-ray energy dispersive analysis studies. X-ray photoelectron spectroscopy for chemical analysis is a relatively new technique for determination of binding energy of electrons in atoms/molecules which depends on distribution of valence charges and thus gives information about the oxidation state of an atom/ion[49]. Electron microscopic observations carried out by Mullen *et al.*[44] revealed the presence of Ag^{2+} as discrete particles at or near the cell wall of both gram-positive and gram-negative bacteria and the presence of silver was confirmed by energy dispersive X-ray analysis (EDAX). Large particles containing gold were localized in *Sargassum natans* cells by EDAX carried out in conjunction with scanning electron microscopy[49]. Figueira *et al.*[78] using the X-ray photoelectron spectroscopy, observed that iron was present in two oxidation states, when brown seaweed *Sargassum fluitans* was exposed to Fe^{2+}, while only Fe^{3+} was present when the biomass was exposed to ferric irons. Further, by FTIR analysis it was confirmed that carboxyl groups were involved in the uptake of both Fe^{2+} and Fe^{3+} and the sulfonate groups were responsible for Fe^{3+} uptake.

Biomass engineering for better biosorbents

As the biosorption process involves mainly cell surface sequesteration, cell wall modification can greatly alter

the binding of metal ions. A number of methods have been employed for cell wall modification of microbial cells in order to enhance the metal binding capacity of biomass and to elucidate the mechanism of biosorption. These modifications can be introduced either during the growth of a micro-organism or in the pregrown biomass. The condition in which micro-organisms grow affects its cell surface phenotype which in turn affects its biosorption potential[27]. Work has been done on the effect of culture conditions of cells on their biosorptive capacity. Luef, Prey and Kubicek[39] reported that *Aspergillus niger* biomass grown in the presence of large amounts of potassium hexacyanoferate from citric acid fermentation plant, exhibited a very high biosorption due to change in cell wall composition. In gram-positive bacteria, Cd^{2+} biosorption was increased by about 14% after addition of nutrients in 2 h of incubation, indicating that some metabolic uptake also occurs besides the passive uptake in bacterial cells. However, in gram-negative bacteria, nutrient addition did not cause any significant increase in Cd^{2+} uptake[25]. In *Phormidium laminosum*, biosorption decreased initially with nitrogen starvation and subsequently increased until it reached the value of nitrogen-sufficient cells[55]. Pregrown biomass could be given several physical and chemical treatments to tailor the metal-binding properties of biomass to specific requirements. The physical treatments include heating/boiling[79], freezing/thawing, drying and lyophilization[3]. The various chemical treatments used for biomass modification include washing the biomass with detergents[78,80], cross-linking with organic solvents[21,80], and alkali or acid treatment[31,80]. The pretreatments have been suggested to modify the surface characteristics/groups either by removing or masking the groups or by exposing more metal-binding sites[34].

Immobilized biomass for bioreactors

From an overview of microbial sorbents and biowaste as sorbent candidate, it can be concluded that laboratory trials do show their potential for commercialization since they possess good metal-binding capacity. Essentially, the main requirement of an industrial sorption system is that the sorbent can be utilized as a fixed or expanded bed and it should not cause much pressure drop across the bed. This will necessitate some degree of pretreatment, sizing, pelleting, chemical modification or immobilization. These are aimed at obtaining a suitable structure for use in a bed reactor and may enhance metal-specific binding sites.

In order to retain the ability of microbial biomass to sorb metal(s) during the continuous industrial process, it is important to utilize an appropriate immobilization technique. The free cells can provide valuable information in laboratory experimentation but are not suited for column packing in industrial applications[75,81]. The free cells generally have low mechanical strength and small particle size and excessive hydrostatic pressures are required to generate suitable flow rates. High pressures can cause disintegration of free biomass. These problems can be avoided by the use of immobilized cell systems[27,82]. Immobilized biomass offers many advantages including better reusability, high biomass loading and minimal clogging in continuous flow systems[21,25]. A number of matrices have been employed for immobilization of cells. One of the matrices that has been used in metal recovery by both viable and non-viable cells is the entrapment in the matrix of insoluble Ca-alginate[83]. Fluidized beds of Ca-entrapped cells of *Chlorella vulgaris* and *Spirulina platensis* were successfully used to recover gold from a simulated gold-bearing process solution containing $AuCl_4$, $CuCl_2$, $FeCl_2$ and $ZnCl_2$ (ref. 68). The Ca-alginate immobilized cells of *Chlorella salina* also showed greater binding of cobalt, zinc and manganese than the free cells[84]. *Rhizopus arrhizus* entrapped on alginate beads was successfully used for the removal of uranium over multiple biosorption and desorption cycles[26]. Accumulation was also dependent on cell density in alginate beads with greater uptake of cobalt at the highest cell densities.

Whole cell immobilization within a polyacrylamide gel also provides a useful laboratory scale system and has been used to biosorb and recover a number of heavy metal(s). Good results have been obtained in the case of polyacrylamide immobilized cells of *Citrobacter* where a very high removal of uranium, cadmium and lead was observed from solutions supplemented with glycerol – $2PO_4$ (ref. 85). *Rhizopus arrhizus* biomass immobilized on polyacrylamide gel was effective in almost completely removing Cu^{2+}, Co^{2+} and Cd^{2+} from synthetic metal solution[26].

Another important matrix being used for immobilization for metal removal is silica. Silica-immobilized preparations offer advantage in terms of reusability and stability. The silica immobilized product is mechanically strong and exhibits excellent flow characterstics[68]. A silica immobilized algal preparation AlgaSORB^R (Bio-Recovery Systems, Inc., Las Cruces, NM 880003, USA) which is being used commercially retains approximately 90% of the original metal uptake efficiency even after prolonged use (> 18 months).

Recent studies have shown the superiority of polyurethane and polysulfone as immobilization support in comparison to polyacrylamide and alginate matrices. Hu and Reeves[86] have reported a novel polyurathane gel bead fabrication technique for immobilizing *Pseudomonas aeruginosa* CSU. Preliminary studies conducted by them revealed that the *P. aeruginosa* CSU biomass immobilized within the polyurethane gel beads were effective in the removal of hexavalent uranium from low concentration acidic waters. Blanco *et al.*[87] immobilized

Phormidium laminosum on polysulfone and epoxy resins. They were successful in reusing the polysulfone immobilized biomass for ten consecutive biosorption/desorption cycles without apparent loss of efficiency after reconditioning it with 0.1 M NaOH. Immobilization of *Citrobacter* biomass in polysulfone matrix increased its metal loading capacity for lead, cadmium and zinc metals[88]. Some matrices used for immobilization of organisms have been summarized in Table 1.

Desorption and reuse of biosorbents

In a number of studies, the biomass has been immobilized using inert solid supports as biofilms[85,89]. These inert matrices include polyvinyl chloride, ZirFon R membrane, glass, metal sheets, plastics, uneven surfaces e.g. wood shavings, clay, sand, crushed rocks and porous materials like foams and sponges.

Biotechnological exploitatation of biosorption technology for removal of heavy metal(s) depends on the efficiency of the regeneration of biosorbent after metal desorption. Therefore non-destructive recovery by mild and cheap desorbing agents is desirable for regeneration of biomass for use in multiple cycles. The efficiency of the desorbing agent or the eluant is often expressed by the S/L ratio, i.e. solid to liquid ratio. The solid represents the solid sorbent (mg dry wt) and the liquid represents the amount of eluant applied (in ml). High values of S/L are desirable for complete elution and to make the process more economical[5,48]. Sometimes metal-selective elution is desirable and can be achieved by the basic understanding of the mechanism involved, in particular, metal sequesteration. It has been observed that for metal ions which show a marked pH dependence in binding to the microbial cells, stripping of bound metal(s) can be accomplished by pH adjustments as has been reported in the case of *Chlorella vulgaris* for desorption of Cu(II), Cr(II), Ni(II), Pb(II), Zn(II), Cd(II) and Co(II) by lowering down the pH to 2.0 (ref. 68). Dilute mineral acids have been used in various studies to remove metal(s) from the loaded biomass[82]. Increasing the acidity generally leads to an effective removal of metal(s) from the biomass.

The physico-chemically sequestered metal to the cell surface can be easily desorbed by EDTA[48,51]. Carbonates and bicarbonates have also been used in the non-destructive recovery of uranium and other metal(s)[48,90]. Kuyucak and Volesky[48] have evaluated the effectiveness of different eluants in stripping cobalt from *Ascophyllum nodosum* and reported that eluants should not effect the cell architecture. $CaCl_2$ (0.05M) in HCl was reported to be the best eluant capable of desorbing about 96% of cobalt at pH 2–3 and it also did not cause any alteration in the cell architecture and its structural materials, whereas all the other eluants including acids, NH_4OH, $KHCO_3$, KCN and EDTA resulted in some changes in the cell architecture.

Commercial biosorbents

Based on the critical analysis of various microbial biomass(es) a few potent metal sequestering biosorbents have been commercialized. A potent algal biosorbent AlgaSorb™ was developed using a fresh water alga *Chlorella vulgaris* to treat wastewater[18]. It can efficiently remove metallic ions from dilute solutions, i.e. 1–100 mg/l and reduces the concentration of metal(s) down to 1 mg/l or even below and its performance was not affected by the presence of calcium and magnesium ions. Another metal sorption agent AMT-BIOCLAIM™ (MRA) has employed *Bacillus* biomass to manufacture granulated material for wastewater treatment and metal recovery[91]. This can accumulate metal cations with efficient removal of more than 99% from dilute solutions. It is non-selective and metal(s) can be stripped using H_2SO_4, NaOH or complexing agents and the granules can be regenerated for repeated use. Bio-Fix biosorbent uses biomass from a variety of sources including cyanobacterium (*Spirulina*), yeast, algae and plants (*Lemna* sp. and *Sphagnum* sp.)[92–94]. The biomass is blended with xanthum and guar gums to give a consistent product and

Table 1. Immobilization matrices used for the study of metal adsorption

Immobilization matrix	Biomass type	Metals adsorbed	References
Calcium alginate	*Chlorella vulgaris* *Spirulina platensis* *Chlorella salina* *Rhizopus arrhizus*	Au, Cu, Fe, Zn, Co, Mn	26, 68, 84
Polyacrylamide gel	*Citrobacter*, *Rhizopus arrhizus*	U, Cd, Pb, Cu, Co, Cd	85
Silica	*Algasorb*	Cu, Ni, U, Pb, Hg, Cd, Zn, As, Ag	68
Polyurethane	*Pseudomonas aeruginosa*	U	86
Polysulfone	*Phormidium laminosum* *Citrobacter*	Pb, Cd, Zn	87, 88

immobilized as beads using polysulfone. Zinc binding to this biosorbent is approximately 4-fold higher than the ion exchange resins. There is variable affinity for different metal(s) $Al^{3+} > Cd^{2+} > Zn^{2+} > Mn^{2+}$ and a much lower affinity for Mg^{2+} and Ca^{2+}. Metal(s) can be eluted using HCl or HNO_3 and the biosorbent can be used for more than 120 extraction–elution cycles.

Conclusions

Thus biosorption offers an economically feasible technology for efficient removal and recovery of metal(s) from aqueous solutions[5,9,28]. The process of biosorption has many attractive features including the selective removal of metal(s) over a broad range of pH and temperature, its rapid kinetics of adsorption and desorption and low capital and operational costs. The biosorbents can easily be produced using inexpensive growth media or obtained as a by-product from some industry. The judicious choice of biosorbent can also out compete the commercial ion exchange resins which have conventionally been used in the removal of metal(s). However, there is a need to have more knowledge of the basic mechanisms involved in order to develop better and effective biosorbents. The major question that still remains to be answered is that although the sorbents are effective, the underlying technology is sound and environmental awareness is growing very fast, still biosorption is not a popular wastewater treatment technology. Critical analysis reveals that not all metal-polluted wastewater-generating industries have the interest or capability to treat effluents. Thus, most of the industries opt for just basic treatment to comply with the legalities. To attract more usage of biosorbent technology, certain strategies have to be formulated to centralize the facilities for accepting the used biosorbent where further processing of the biosorbent can be done to either regenerate the biomass and then convert the recovered metal into usable form. This will further require an interdisciplinary approach with integration of metallurgical skills along with sorption and wastewater treatment to develop biosorption technology for combating heavy metal pollution in aqueous solutions.

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Received 8 August 1999; revised accepted 3 December 1999